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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	**Carl G. Hellerqvist**		
Application No.:	**09/776,865**	Art Unit:	**1642**
Filed:	**February 2, 2001**	Examiner:	**Stephen L. Rawlings**
For:	**Methods for Preventing or Attenuating Pathoangiogenic Conditions**		

SECOND DECLARATION OF DR. CARL G. HELLERQVIST UNDER 37 C.F.R. §1.132

I, Carl G. Hellerqvist, Ph.D., do hereby declare:

1. I am an expert in the field of the invention. My qualifications are of record on the U.S. Patent Application Serial Number 09/776,865, filed February 2, 2001, (hereinafter referred to as "the present application").

2. I am a sole named inventor on the present application. I am familiar with the Office Actions mailed by the United States Patent and Trademark Office in the present application on July 16, 2003 and July 26, 2004, the Final Office Action mailed by the United States Patent and Trademark Office on February 24, 2005, and the Non-Final Office Action mailed September 6, 2005.

3. As one of ordinary skill in the art in the field of the invention, I declare that, in the field of tumor angiogenesis studies, observations in appropriately selected mouse models reasonably correlate with the observations in other mammals, such as humans. Mouse animal models used in the examples of the present application are selected so that they are reasonably correlating with human pathological angiogenesis for the purpose of

demonstration of the ability of Group B ß-hemolytic *Streptococci* ("GBS") toxin receptors or immunogenic fragments thereof to attenuate pathological angiogenesis, in particular, the pathological angiogenesis associated with cancer in a mammal, including a human. This is because the methods disclosed and claimed in the present application affect homologous targets in pathological vasculature, common to the animals and the humans.

4. I discovered that Group B Streptococcal toxin, GBS toxin/CM101 is an agent that induced respiratory distress in human neonates by binding to neonatal vasculature. I did so by using a sheep model because GBS is a sheep pathogen. The damage induced by GBS to sheep lung vasculature correlated with damage to human neonatal lung vasculature. See, for example, the present application, p. 5, lines 5-26; Fu *et al.*, *Clin. Cancer Research*, v. 7, pp. 4182-4194 (2001; of record in the present application) and references cited therein on p. 4182, second column. Similarly to human neonates, mice neonates are susceptible to GBS infections. Madoff L.C., *et al.*, *J. Clin. Invest.*, v. 94, pp. 286-292. (1994; Exhibit A). Accordingly, GBS-induced damage in neonatal mouse lung vasculature is expected to correlate with the damage in neonatal humans. While not wishing to be bound by the following hypothesis, I suggest that tumors originate from cells reverting back towards an embryonic stage. Therefore, tumor-recruited vasculature in humans and mice may also have neonatal characteristics, such as GBS binding. Applicants generated antibodies to GBS toxin/CM101 and showed by immunohistochemistry that CM101 bound to human and mouse tumor vasculature but not to normal vasculature in humans, mice. See, for example, Yan *et al.*, *Angiogenesis*, 2: 219-233 (1998;Exhibit B); Wamil *et al.*, *J. Can. Res. Clin. Oncol.*, v. 123, pp. 173-179 (1997; Exhibit C). Thus, mouse and human tumor vasculature possess similar GBS toxin-binding markers.

5. I used a nude mouse model implanted with a human carcinoma of the breast in order to conduct preclinical studies of the cancer-attenuating properties of CM101. Infusing CM101 every other day for twenty-one days lead to a 60% decrease in tumor volume. See, for example, US Patent No. 5,010,062 (Example 2; Exhibit D). I demonstrated that CM101

induced a Complement C3 activated cytokine driven inflammatory response targeting the tumor vasculature. See, for example, Yan *et al.* (1998 Exhibit B).

The above results from the mouse models correlated with the results of clinical testing in human patients. Patients responded to catalytic amounts of GBS toxin/CM101 with a complement activated cytokine driven inflammatory response that targeted the tumor vasculature and induced pain at the sites of tumors. See, for example, Wamil *et al.* (1997 Exhibit C); DeVore *et al.*, *J. Clin. Can. Res.* v. 3, pp. 365-372 (1997; Exhibit E). The observations of induced inflammation, breakdown of immunoprivilege in the tumor, and tumor cell apoptosis in human patients were confirmed in a mouse model. See Yakes *et al.*, *Cancer Research* v. 60, pp. 5740-5746 (2000; Exhibit F). Thus, I demonstrated the correlation of the effects of GBS toxin administration in mouse cancer models and human cancer patients.

6. By cloning and identifying HP59, the target protein for GBS toxin/CM101, in humans and sheep, and by immunohistochemical studies in mice, I showed the existence of a conserved molecular marker for neonatal and pathologic vasculature in mammals. See Fu *et al.* (2001). I also showed that HP59 is present in the tumor vasculature independently of site and type. See Table 1 in Fu *et al.* To my knowledge, all antibodies generated to the human and sheep HP59 analogue cross-react with pathologic vasculature in mice rats and pigs. Thus, HP59 is a target in pathologic vasculature which is common to humans, mice rats and pigs.

7. I showed in Fu *et al.* (2001) and in the present application that immunization with HP59 and Sp55 peptides attenuated tumor growth in a mouse animal model by inhibiting pathoangiogenesis and vasculogenesis. The HP59 derived vaccines are able to inhibit pathologic angiogenesis and to generate a cellular immune response. I further demonstrated that the immunized mice that survived repeated intravenous melanoma injections developed a cellular immune response to the vascular target. This immune response led to attenuation of the pathologic angiogenesis. White blood cells (WBC) were

isolated from the tumor resistant mice or control mice and infused in naïve mice carrying Lewis lung tumor or melanoma in a cutaneous window model. WBC from control mice had no effect on the naïve mice, whereas administration of WBC from the tumor-challenged immunized mice to model animals attenuated pathological vasculature in the model animals. See Figure 1 - Exhibit G. HP59 target is unique to pathologic vasculature and common to the pathologic vasculature of at least humans, sheep and mice. Thus, I identified HP59 as a target for attenuating cancer-associated pathologic angiogenesis in various mammals.

8. I declare that the publications cited by the Examiner in the last Office Action predominantly deal with the limitations of mouse models for predicting clinical outcomes during development of **drugs targeting various tumor-specific targets, and not treatments directed at pathological vasculature-specific targets.** For example, Wang *et al.* (2001) deals with anti-cancer vaccines against tumor-specific antigens. Accordingly, the cited publications are not relevant to the methods and compositions of the present application.

Genetic and phenotypical diversity of the tumor tissues makes it difficult to target tumor-specific targets. This genetic and phenotypical diversity of the tumor tissues negatively influences the correlation between mouse models and humans when tumor targets are affected. See, for example, Axelson *et al.*, *Semin. Cell. Dev. Biol.* v. 16, 554-63 (2005; Exhibit G):

> "Histopathological examination of solid tumors frequently reveals pronounced tumor cell heterogeneity with regards to cell organization, cell morphology, cell size, nuclei morphology, etc. Analyses of gene expression patterns by immunohistochemistry or in situ hybridization techniques further strengthen the actual presence of phenotypic heterogeneity, often demonstrating substantial diversity within a given tumor. The molecular mechanisms underlying the phenotypic heterogeneity are very complex with genetic, epigenetic and environmental components."

9. In contrast to the limited correlation between animal models and humans for tumor-specific targets, **one of ordinary skill in the art expects reasonable correlation between animal models and humans when targeting cancer-associated pathologic vasculature, provided that a vascular target is common in a model animal and a human.** See, for example, Klelland *et al.* (2004) (cited by the Examiner). Klelland *et al.* recognizes mouse xenograft models as valuable, but cautions against using them when testing compounds that target tumor vasculature because the vasculature in the xenograft is of mouse origin, and may therefore lack a target protein found in a human (see p. 834 in Klelland *et al.*). In direct contrast to the situation described in Klelland *et al.*, in the present application, I selected a mouse model exactly because mouse vasculature possesses the same target as human and sheep vasculature. Thus, according to Klelland *et al.* criteria, mouse models reasonably correlate with human for the purpose of observing inhibition by angiogenesis by administering the compositions of the present application that target common targets in human and mouse vasculature..

10. At least one reason for the expected reasonable correlation between animal models and humans when testing the therapies that target pathologic vasculature is that such vasculature is relatively genetically and phenotypically homogenous, in contrast to tumor tissues. The advantages of targeting tumor vasculature due to "genetic stability of target cells" are supported in Jain, *Oncology*, v. 19 (4 Suppl. 3), pp. 7-16. (2005; Exhibit H). The existence of unique molecular entities in pathologic vasculature that may represent potential therapeutic targets is also supported in St. Croix *et al.*, *Science* , v. 289, pp. 1197-202 (2000; Exhibit I):

> "To gain a molecular understanding of tumor angiogenesis, we compared gene expression patterns of endothelial cells derived from blood vessels of normal and malignant colorectal tissues. Of over 170 transcripts predominantly expressed in the endothelium, 79 were differentially expressed, including 46 that were specifically elevated in tumor-associated endothelium. Several of these genes encode extracellular matrix proteins, but most are of unknown function. Most of these tumor endothelial markers were expressed in a wide range of tumor types, as well as in normal vessels associated with wound healing and corpus luteum formation. These studies demonstrate that

> tumor and normal endothelium are distinct at the molecular level, a finding that may have significant implications for the development of anti-angiogenic therapies."

I demonstrate that HP59 homologous protein, are expressed in cutaneous wounds also in pigs and that CM101 treatment accelerates wound healing by blocking pathologic angiogenesis See Nanney LB, et al Angiogenesis. 2001-4(1)61-70 Exhibit J)

> "The immunolocalization HP59 in the microvessels of the cutaneous wound bed in control but not in CM101 wounds of untreated pigs suggests that CM101 inhibits the pathologic inflammatory angiogenesis accompanying the normal granulation processes. The net biological effect of inhibited inflammatory pathoangiogenesis is a diminished, suggested and purely physiologic, microvascular bed which translates into an enhanced rate of epithelial resurfacing and therefore an overall accelerated rate of wound repair."

11. An example of the successful application of targeting pathologic vasculature are anti-cancer therapies targeting vascular endothelial growth factor (VEGF). The mouse models used with anti-VEGF antibodies correlated reasonably in colon and breast cancer. See Ferrara *et al., Biochem. Biophys. Res. Commun.* v. 333, pp. 289-91 (2005; Exhibit K):

> "VEGF plays an essential role in developmental angiogenesis and is important also for reproductive and bone angiogenesis. Substantial evidence also implicates VEGF as a mediator of pathological angiogenesis. Anti-VEGF monoclonal antibodies and other VEGF inhibitors block the growth of several tumor cell lines in nude mice. Clinical trials with VEGF inhibitors in a variety of malignancies are ongoing. Recently, a humanized anti-VEGF monoclonal antibody (bevacizumab; Avastin) has been approved by the FDA as a first-line treatment for metastatic colorectal cancer in combination with chemotherapy. Furthermore, VEGF is implicated in intraocular neovascularization associated with diabetic retinopathy and age-related macular degeneration."

12. In the present application, my methods and compositions target a protein common to pathologic vasculature of various types of tumors and shared by at least humans,

mice, rats and pigs. I elucidated the mechanism of action of GBS toxin during angiogenesis, and, in particular how CM101 binds to HP59 and induces an inflammatory cascade, which leads to infiltration of granulocytes, lymphocytes and lymphocytes and destruction through apoptosis of the pathologic conditions. See Yakes *et al.*, *Cancer Research* v. 60, pp. 5740-5746 (2000; Exhibit F). The attenuation of cancer-associated angiogenesis in the mouse models used in the working examples of the present application is reasonably correlating with the human pathologic angiogenesis associated with conditions such as cancer, due to the presence of common CM101 target proteins in mice and humans and common mechanism of action of my claimed methods.

13. I declare further that all statements made herein are of my own knowledge and are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine, or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of any patent issuing on this application.

Signature

Carl G. Hellerqvist, Ph.D.
Name

2/7/2006
Date